WO 2005/061698 PCT/NL2004/000911

37

Claims

- 1. A virion of a pneumovirus comprising a viral genome that has a mutation in a gene coding for a protein that is essential for infectivity of the pneumovirus, whereby the mutation causes a virus produced from only the viral genome to lack infectivity, and whereby the virion comprises the protein in a form and in an amount that is required for infectivity of the virion.
- A virion according to claim 1, whereby the pneumovirus is a Respiratory
 Syncytial Virus.
 - 3. A virion according to claims 1 or 2, whereby the gene codes for a G attachment protein.
- 4. A virion according to any one of claims 1-3, whereby the mutation causes the virus produced from only the viral genome to lack the protein.
 - 5. A virion according to claim 1, whereby the mutation comprises deletion of the sequence coding for the protein.

20

25

30

- 6. A method for producing pneumoviral virions, the virions comprising a viral genome that has a mutation in a gene coding for a protein that is essential for (in vivo) infectivity of the pneumovirus, whereby the mutation causes a virus produced from only the viral genome to lack infectivity, and whereby the virion comprises the protein in a form and in an amount that is required for infectivity of the virion, the method comprising the steps of:
- (a) infecting a culture of a first host cell with a pneumovirus comprising a viral genome that has the mutation, whereby the host cell comprises an expression vector which directs expression in the host cell of the protein in a form and in an amount that is required for infectivity of the virion; and,
- (b) recovery of the virions from the infected host cell culture.

WO 2005/061698 PCT/NL2004/000911

7. A method according to claim 6, whereby the pneumovirus that is used to infect the culture of a first host cell culture, is produced the method comprising the steps of:

38

- (a) providing to a second host cell one or more expression vectors which direct expression in the host cell of:
- i) a viral genomic RNA that has a mutation in a gene coding for a protein that is essential for (in vivo) infectivity of the pneumovirus, whereby the mutation causes a virus produced from only the viral genome to lack infectivity;
 - ii) a pneumoviral polymerase enzyme complex and optionally one or more further viral proteins; and,
- 10 (b) culturing the second host cell whereby the virions are produced.
 - 8. A method according to claim 7, which further comprises amplifying the virions produced by the second host cell by one or more further cellular infection steps employing host cells which are the same or different from the second host cell.

15

20

- 9. A method according to claims 7 or 8 wherein the viral genomic RNA is transcribed from a viral DNA copy that is under the control of a bacteriophage DNA-dependent RNA polymerase promoter and whereby the host cell is provided with an expression vector which directs expression in the host cell of the bacteriophage DNA-dependent RNA polymerase.
- 10. A method according to claim 9 whereby the bacteriophage DNA-dependent RNA polymerase is the T7, T3 or SP6 polymerase.
- 25 11. A method according to any one of claims 7-10, whereby the pneumoviral polymerase enzyme complex at least includes the L, P, N proteins.
 - 12. A method according to any one of claims 7-11, whereby one or more further viral proteins is a pneumoviral matrix membrane protein, preferably the M2-1 protein.

30

13. A method according to any one of claims 6-12, whereby the pneumovirus is a Respiratory Syncytial Virus.

WO 2005/061698 PCT/NL2004/000911

14. A method according to any one of claims 6-13, whereby the gene coding for the protein that is essential for infectivity is a gene coding for a G attachment protein.

39

- 15. A composition comprising a virion as defined in any one of claims 1-5, or obtainable in a method as defined in any one of claims 6-14, and a pharmaceutically acceptable carrier.
 - 16. Use of a virion as defined in any one of claims 1-5 for the manufacture of a medicament for the prevention or treatment of a pneumoviral infection.

17. A use according to claim 16, the medicament is a preparation for intranasal administration.

10

- 18. A method for the prevention or treatment of a pneumoviral infection, the method comprising the step of administering to a subject a composition comprising a virion as defined in any one of claims 1-5, in an amount effective to prevent or treat the infection.
- 19. A method according to claim 18, wherein the composition is administered intranasally.

